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Urban wastewater treatment by seven species of microalgae and an algal bloom: Biomass production, N and P removal kinetics and harvestability

Fatima Zahra Mennaa [a, *], Zouhayr Arbib [b, c], José Antonio Perales [b]

[a] Department of Environmental Technologies, Instituto de Desarrollo Tecnológico & Industrial de la Bahía de Algeciras, Escuela Politécnica Superior de Algeciras, Universidad de Cádiz, Avda. Ramón Puyol s/n, 11202 Algeciras Cadiz, Spain
[b] Department of Environmental Technologies, Centro Andaluz de Ciencia y Tecnología Marinas (CACYTMAR), Campus de Excelencia Internacional del Mar (CEIMAR), Campus Universitario de Puerto Real, Universidad de Cádiz, 11510 Puerto Real, Cadiz, Spain
[c] Aqualia Gestión Integral del Agua, SA, Spain

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ABSTRACT

This study evaluates the capacity of seven species and a *Bloom of microalgae* to grow in urban wastewater. Nutrient removal kinetics and biomass harvesting by means of centrifugation and coagulation –flocculation-sedimentation have been also tested. Results show that the best biomass productivities ranged from between 118 and 108 mgSS L^{-1} d^{-1} for the *Bloom (Bl)* and *Scenedesmus obliquus (Sco)*. Regarding nutrient removal, microalgae were able to remove the total dissolved phosphorus and nitrogen concentrations by more than 80% and 87% respectively, depending on the species tested. The final total dissolved concentration of nitrogen and phosphorus in the culture media complies with the European Commission Directive 98/15/CE on urban wastewater treatment. Regarding harvesting, the results of coagulation–flocculation sedimentation using a 60 mg L^{-1} dose of Ferric chloride were similar between species, exceeding the biomass removal efficiency by more than 90%. The results of centrifugation (time required to remove 90% of solids at 1000 rpm) were not similar between species, with the shortest time being 2.9 min for *Sco*, followed by the *bloom* (7.25 min). An overall analysis suggested that *the natural bloom* and *Scenedesmus obliquus* seem to be the best candidates to grow in pre-treated wastewater, according to their biomass production, nutrient removal capability and harvestability.

1. Introduction

The aquatic ecosystem is severely affected by anthropogenic activity from urbanization, industrialization, agriculture and other alterations. All of these activities increase nutrient inputs into water bodies, especially nitrogen and phosphorus. A European Commission Directive (98/15/EC, 1998) defined Eutrophication as enrichment of water by nutrients, especially compounds of nitrogen and phosphorus, causing accelerated growth of algae and higher forms of plant life and thereby producing an undesirable disturbance to the balance of organisms and the quality of the water concerned.

Eutrophication has led to the development of nutrient removal processes from wastewaters by means of physical, chemical and biological processes (de-Bashan and Bashan, 2004; Ahn, 2006; Ghafari et al., 2008). These processes are based on biological processes which include different combinations of anaerobic, aerobic, and anoxic zones with internal recirculation such as Bardenpho, A2O, UCT, and their modifications. The main disadvantages of these advanced technologies are high costs, complex operation and great volume of waste sludge production (von Sperling and Augusto de Lemos Chernicharo, 2002).

Microalgae has demonstrated great potential as an alternative to advanced biological nutrient removal (BNR) (Mallick, 2002; Olguín, 2003; Órpez et al., 2009; Arbib et al., 2012; Ruiz et al., 2013a,b) because it can reduce energy costs. The process does not require the addition of chemicals, it is simplified by not needing numerous tanks for operations and internal recycling, it can simultaneously deliver nutrient removal and reduce the CO_2 footprint and it

* Corresponding author.
E-mail addresses: fatima.mennaa@uca.es (F.Z. Mennaa), zouhayr.arbib@fcc.es (Z. Arbib), joseantonio.perales@uca.es (J.A. Perales).

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generates oxygenated treated water to the receiving bodies. Additionally, microalgae sludge has ample post-application potential; it could be a source of high-value products (Mallick, 2002) for use as biofuels and bioproducts.

One of the main challenges in microalgae biotechnology, and especially for wastewater treatment, is the efficient and reliable separation of microalgae from the culture broth. It is worth mentioning that the harvesting step is one of the bottlenecks of microalgae biotechnology. Depending on species, cell density, and culture conditions, harvesting algal biomass has been estimated to contribute 20–30% to the production costs (Gudin and Therpenier, 1986; Rodolfi et al., 2009; Molina Grima et al., 2003). In order to concentrate large microalgae culture volumes, a suitable harvesting method may involve one or more steps and be achieved in several physical, chemical, or biological ways (Mata et al., 2010). Experience has demonstrated that a universal harvesting method does not exist (Shelef et al., 1984). This is still an active area for research, with it being possible to develop an appropriate and economical harvesting system for any species of algae (Molina Grima et al., 2003; Mata et al., 2010). For low-value bulk products, both the investment and the operational costs must be drastically reduced to make commercial production viable (Wijffels et al., 2010).

Strategies for harvesting microalgae include flotation, sedimentation, filtration and centrifugation (Shelef et al., 1984; Molina Grima et al., 2003; Danquah et al., 2009). These processes are supplemented by coagulation and/or the flocculation method, either through the addition of chemical coagulants and flocculants or through autoflocculation by raising the pH to values above 10.

The aim of this work is to study the harvestability by means of centrifugation, flocculation or settling of seven species of microalgae; *Ankistrodesmus falcatus (Af)*, *Scenedesmus obliquus (Sco)*, *Chlorella kessleri (Ck)*, *Chlorella Vulgaris (Cv)*, *Chlorella sorokiniana (Cs)*, *Botryococcus braunii (Bb)*, *Neochloris oleabundans (No)* and a *natural bloom* cultured in urban wastewater, as well as to compare their growth and nutrient removal kinetics.

2. Material and methods

2.1. Microorganism and culture conditions

The microalgae used were *Chlorella vulgaris* (SAG 211-12), *C. kessleri* (SAG 211-11), *C. sorokiniana* (SAG 211-8k), *B. braunii* (SAG 30.81) *S. obliquus* (SAG 276-10) and *A. falcatus* (SAG-202-2) from the Culture Collection of Algae (SAG), Göttingen University (Germany), and *N. oleabundans* (UTEX-1185) from the Culture Collection at the University of Texas (Austin, USA). A *natural algal bloom* obtained from wastewater of the Wastewater Treatment Plant (WWTP) of the city of "La Línea de la Concepción" (36° 11′ 40″ N 5° 20′ 55″ W Cadiz, Spain). This bloom was obtained by means of incubating settled urban wastewater under the same conditions as the rest of the microalgae inocula. These were incubated in synthetic culture medium Combo double concentrated (Kilham et al., 1998) at 20 ± 3 °C under a 14/10 h L/D (light/dark) cycle. The inoculum cultures were maintained in exponential growth adding 200 ml (every 2 or 3 days) of synthetic culture medium scaling up the volume up to 2 L of batch reactor. As inoculum for the experiments, a concentrate of microalgae from centrifugation was used, in order to avoid nutrient enrichment.

2.2. Experimental set-up

The experiments were conducted in batch photobioreactors on a laboratory scale by using 2000 ml borosilicate Pyrex bottles (12.5 cm diameter × 14.5 cm height) sealed with hydrophobic cotton. Air was bubbled into the bottom of the photobioreactor at a flow rate of 1 L min^{-1}. Aeration provided CO_2, prevented cells sedimentation and kept the reactor in completely mixed conditions.

The temperature was maintained at 20 ± 3 °C. The cultures were lit by two fluorescent lamps (1 PHILIPS Master TLD 58W/840 Cool White and 1 SYLVANIA Grolux F58W/GRO-T8 Daylight) placed horizontally and parallel to the front side of the photobioreactor. The incident light intensity was of 90 μmol m^{-2} s^{-1} measured with a PAR Hansatech, Quantitherm light meter.

2.3. Culture medium

The experiments were carried out using urban wastewater as a culture medium, supplied by a town in the south of Spain (La Linea de la Concepcion, 64.704 inhabitants). This culture medium was unfiltered pre-treated wastewater (screened plus sand and grease removed). Wastewater characterization used for the tests was: COD = 384 mgO_2 L^{-1}; Total P = 12.70 mg L^{-1}; Total N = 54.58 mg L^{-1} and SS = 131.25 mgSS L^{-1} (suspended solids of settled water after 24 h).

2.4. Analytical methods

Temporal evolution of the microalgae biomass was measured daily by means of the algae dry weight, determined gravimetrically as suspended solids according to Standard Methods 2540-D (APHA-AWA-WPCF, 1992): Liquid samples for nutrient consumption analysis were taken daily from each reactor. Samples were analysed after filtration through a filter of 0.7 μm to separate solids. A Merck-Spectroquant analytical kit was used to analyse nitrate Cod. 1.14773.0001 (Merck). Phosphate concentration analysis was performed according to standard methods 4500-P E (APHA-AWWA-WPCF, 1992). Total nitrogen (TN) and total phosphorus (TP) were determined, based on the method proposed by Köthe and Bitsch (1992), mixing 10 mL of the sample and 1.5 microspoon of OXI-SOLV® (Merck KGaA, Darmstadt, Germany), incubating it at 100 °C for 60 min and then cooling it at room temperature. Once samples were completely oxidized, nitrate and phosphate determinations were performed to determine total nitrogen and total phosphorus concentrations respectively.

2.5. Statistical analysis

Data was fitted to the corresponding kinetic models, minimizing the sum of squared residuals using the Microsoft Excel Solver tool, which uses a Non-linear programming algorithm called GRG2, an implementation of the generalized reduced gradient algorithm (Lasdon et al., 1978). The fitting parameters used were: Precision: 10–15; Tolerance: 5%; Convergence: 10^{-4}.

2.6. Biomass growth kinetics

The Verhulst logistic kinetic model (Verhulst, 1838) was used to model the evolution of the experimental biomass concentration in the reactors. The model is a substrate-independent equation and can accurately describe biomass growth in the different culture conditions which occur in many batch bioreactors (Gong and Lun, 1996). According to the model, microbial growth can be expressed as a sinusoidal curve, as described by Equation (1).

$$\frac{\delta X(t)}{\delta t} = \mu X(t)\left[1 - \frac{X(t)}{X_{max}}\right] \quad (1)$$

Integrating this equation, we get Equation (2), where, μ (μ_{max}) is the maximum specific growth rate (d^{-1}), X_{max}, X_0 and X are the

concentrations of biomass (mg L^{-1}) at operating times equal to infinity, zero and t respectively.

$$X = \frac{X_0 X_{max} e^{\mu t}}{X_{max} - X_0 + X_0 e^{\mu t}} \quad (2)$$

Productivity is an important parameter to consider in the technology for cultivating microalgae, as it shows the capacity of a reactor to produce biomass under specific operating conditions and defined as the biomass produced per reactor volume and per unit time. This parameter can be calculated from the kinetic parameters of Verhulst model (Ruiz et al., 2013a,b) as:

$$Productivity = \frac{\mu \cdot (0.9 \cdot X_{max} - 1.1 \cdot X_0)}{Ln\left(\frac{9 \cdot (X_{max} - 1.1 X_0)}{1.1 \cdot X_0}\right)} \quad (3)$$

2.7. Nutrient removal kinetics

Equation (4) has been used to describe the experimental data for total nitrogen and total phosphorus (Ruiz et al., 2013a,b).

$$S = \frac{\left(\frac{X_0}{Y_0} + S_0\right)(S_0 - S_{na}) - S_{na}\left(S_0 - \left(\frac{X_0}{Y_0} + S_0\right)\right)e^{\mu t}}{(S_0 - S_{na}) - \left(S_0 - \left(\frac{X_0}{Y_0} + S_0\right)\right)e^{\mu t}} \quad (4)$$

Where:

X_0 = Initial concentration of biomass (mg SS L^{-1})
$1/Y_0$ = Nutrient content of the biomass used for inoculation (mg N or P mg SS^{-1})
S_0 = Initial dissolved nutrient concentration (mg N or P L^{-1})
S = Dissolved nutrient concentration at an instant t (mg N or P L^{-1})
S_{na} = Unassimilated dissolved nutrient concentration (mg N or P L^{-1})
μ = the maximum specific growth rate (μ_{max}) of the microalgae (d^{-1}).

To apply the model, experimental data for dissolved nutrient concentration were used as S value, X_0 value used was that from the biomass growth modelling described in the previous paragraph, so the adjustable factors in the model were S_0, S_{na}, $1/Y_0$ and μ, but with the following restrictions (S_{na} value should be greater or equal to 0 and $1/Y_0$ should be in the interval of 0.01–0.1 mgN mg SS^{-1} (Syrett, 1981; Ruiz et al. 2014), and 0.001 to 0.01 mgP mg SS^{-1} (Goldman, 1980; Ruiz et al. 2014).

The consumption rate of total nitrogen and phosphorus (dN or dP/dt; mg L^{-1} d^{-1}) was calculated with this equation:

$$CR_{max} = \left(\frac{-dS}{dt}\right)_{max} \approx \left(\frac{-\Delta S}{\Delta t}\right)_{max} = \left(\frac{S_t - S_{t+\Delta t}}{\Delta t}\right)_{max} \quad (5)$$

Where:

CR_{max} = the maximum daily consumption rate of total dissolved nitrogen or phosphorus (mg L^{-1} d^{-1})
S = Substrate concentration of dissolved nitrogen or phosphorus at a specific time t (mg L^{-1})

2.8. Harvesting testing

Once biomass concentration in each test reached the stationary phase, the reactor medium was subjected to two harvesting experimental procedures:

2.8.1. Flocculation test

2.8.1.1. Procedure validation. The jar-test was selected as the standard treatment in order to study the effect of various dosages of a flocculant on the microalgae flocculation process and compare them with the micro jar-test procedure using a tube rotating shaker. *C. vulgaris* (SAG 211-12) was grown in a synthetic culture medium Combo (Kilham et al., 1998). Flocculation experiments were carried out in the stationary growth phase. All experiments were carried out at room temperature using two different apparatuses:

i) **Jar-Test:** a six unit stirrer apparatus (OVAN Flocculators Jar test JT60E) provided with six one litre graduated beakers (Scott Duran 105 × 145 mm). The stirrer blades (65 × 24 mm) were made of Perspex and immersed at 3 cm from the bottom of the jars. Each 0.7 L sample was doused with predetermined concentration of flocculant from freshly prepared stock solution. A stock solution was prepared by dissolving 13.8 g of Ferric chloride (salt ferric chloride, $FeCl_3$: Iron (III) Chloride Anhydrous, 97% PS) into 1000 mL distilled water.
ii) **Tube rotating shaker apparatus:** In sterile plastic tubes with screw caps (size 16 × 102 mm, capacity 12 mL) with 10 mL testing volume with two replications containing microalgae and flocculant of different jars.

Six different dosages were used (0, 10, 20, 40, 80, 100 mg $FeCl_3$ L^{-1}). The initial sample pH was 8.26. Then, the flocculant addition samples were stirred at 80 rpm for 2 min (jar test apparatus) and 15 s at full speed 40 Hz (tube vibrating shaker) to ensure that the coagulant is completely dissolved (Fig. 1). Following this, the stirring was reduced to gentle agitation at 20 rpm for 30 min in test-jar device and Tubes Rotating Shaker to promote flocs formation. At the end of the 30 min the stirrers were turned off to allow settling for 30 min in both apparatus. Immediately afterwards, samples were taken from the 0.65 L level in jars and 9.5 mL in tube (this was measured at half the height of the clarified culture) (Bilad et al., 2012) for determination of algal residues after flocculation and sedimentation.

2.8.1.2. Flocculation test. The effect of various dosages of a given flocculant on microalgae flocculation was studied on a small scale and at room temperature by means of a micro-jar test using a tube rotating shaker apparatus (Heidolph Shaker REAX 2. 20–100 rpm). The tube rotating shaker has capacity for 20 tubes of 10 mL (16 mm × 100 mm), and turns them around a perpendicular axis. A 9 mL sample was dosed to each tube to which was previously added 1 mL of coagulant (salt ferric chloride, $FeCl_3$: Iron (III) Chloride Anhydrous, 97% PS) from the corresponding freshly prepared stock solution (600 mg L^{-1} of $FeCl_3$). The typical coagulant dosage of $FeCl_3$ for wastewater coagulation ranged between 35 and 150 mg L^{-1} (Eckenfelder, 1989). In a previous screening jar test using Cv cultivated in wastewater, $FeCl_3$ concentration between (0–100 mg L^{-1}) was tested. 60 mg L^{-1} $FeCl_3$ was the minimum dosage to reach 90% of biomass removal. After sample and coagulant addition, tubes were stirred at 80 rpm for 15 s, followed by a flocculation set; slow mix (20 rpm) for 30 min. Finally, the tubes were left to settle for 30 min (Sukenic et al., 1988). Immediately after coagulation, flocculation and sedimentation, a sample of the solution was pipetted from a fixed height (10 mm) from the surface of the water level and the optical density at 680 nm was determined to calculate the removal efficacy. Biomass removal efficiency (BRE) was calculated by measuring optical density and using the

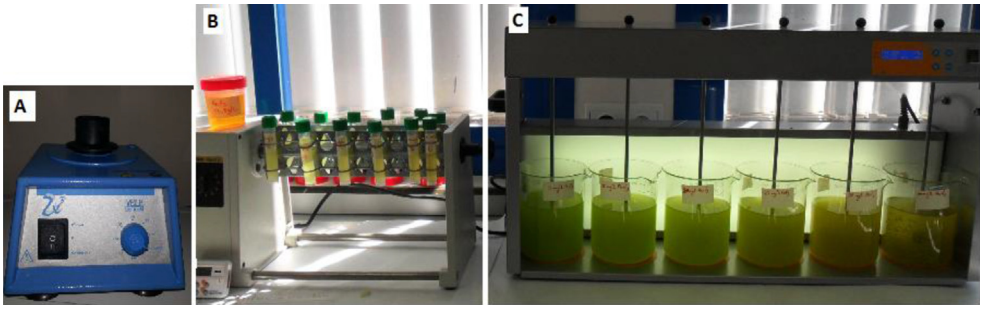

Fig. 1. Flocculation *Chlorella vulgaris*. A: Tube vibrating shaker apparatus; B: Tube rotating shaker apparatus and C: Jar-test apparatus.

following Equation (1) (Garzon-Sanabria et al., 2012).

$$BRE = \left(1 - O.D_{680f} \Big/ O.D_{680i}\right) \times 100 \quad (6)$$

Where $O.D_f$ is the optical density after 30 min settling and $O.D_i$ is the initial optical density.

2.8.2. Centrifugation test

The centrifugation experiments were carried out in tubes of 10 mL capacity. The equipment used was a centrifuge (HETTICH ZENTRIFUGEN EBA 20) with a capacity of 8 × 15 mL tubes and a rotation frequency of 1000–6000 rpm (rotation radius = 231 mm). Laboratory centrifugation tests were conducted at different times (1, 2, 4, 8 and 10 min) at 1000 rpm and at room temperature. At the end of centrifugation, biomass concentration in supernatant was determined in order to obtain the efficacy reached (BRE), and this was calculated in the same way as the flocculation.

3. Results and discussion

3.1. Biomass growth rate

Fig. 2 shows the evolution of biomass concentrations of the seven microalgae species and the natural bloom cultured in wastewater (WW). A typical evolution in four phases of a batch culture can be observed: (1) there is an initial period of physiological adjustment (lag-phase period for adaptation to the wastewater condition) due to changes in nutrient or culture conditions, this phase in all the tests was found to be short (<10 h), except for *Af* for which the lag phase was longer (≈25 h) while the bloom showed the shortest lag phase (<6 h); (2) an accelerated growth phase where the cells begin to grow and multiply once they are adapted to the new environment; (3) an exponential growth phase characterized by cell doubling and biomass growth at a constant rate, this period is species-dependent, as can be observed; and finally (4) a stationary phase where biomass growth rate is often practically zero as a result of the lack of a fundamental growth element (Nitrogen, phosphorus, light, etc…). This stationary phase was not reached in *Af* test as it was stopped after 40 days of cultivation.

Regarding pH evolution, all the tests presented a similar pattern. At the beginning of the experiments the pH was about 7.06 in all the reactors and this increased, reaching average pH values between 9.69 and 10.30, after about 3 days of operation. These values were almost constant throughout the experiment. The pH rise is associated with the CO_2 consumption by microalgae that alter the balance of inorganic carbon speciation in the media towards proton depletion.

The experimental biomass evolution fits the Verhulst model very well (see lines in Fig. 2), with the proportion of variance explained by the equation being higher than 98% in all the tests. Table 1 presents the kinetic growth parameters obtained.

Regarding the specific growth rate obtained, there were significant differences between the different strains tested, with values ranging from 0.1 d^{-1} to 0.52 d^{-1} (Table 1). This huge difference is related to the different ability of each strain to acclimate to the culture medium and/or culture condition. Four different groups can

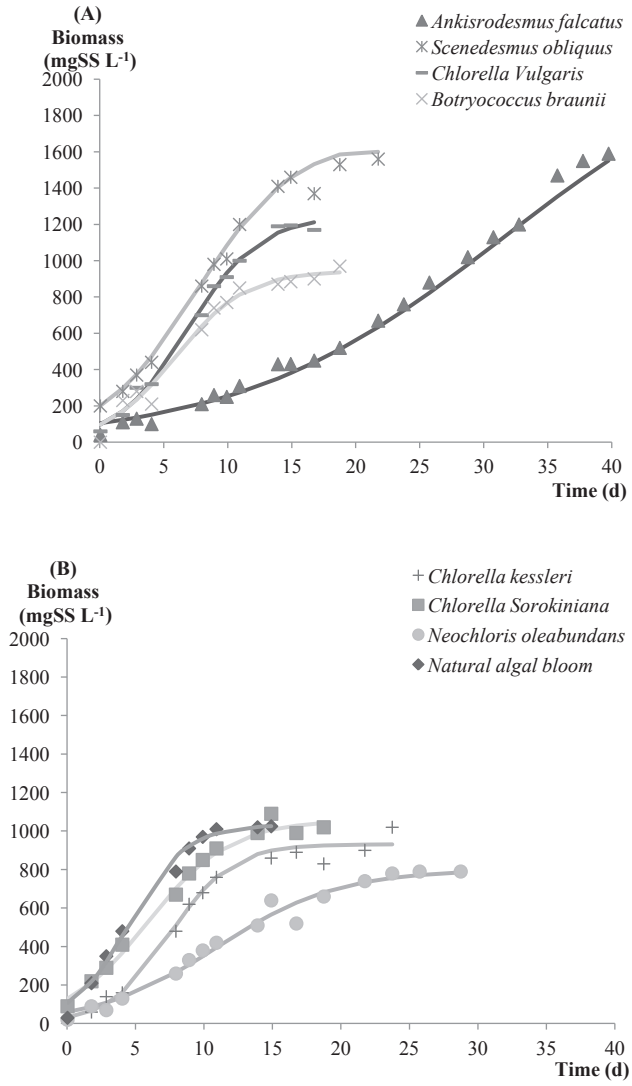

Fig. 2. (A) and (B) Experimental and predicted biomass evolution of *Cv*, *Ck*, *Cs*, *Bb*, *Sco*, *Af*, *No* and *Bloom* (lines represent the values predicted by the Verhulst logistic model).

Table 1
Verhulst kinetic growth parameters.

Parameters	Af	Sco	Ck	Cv	Cs	Bb	No	Bloom
X_o (mg SS L^{-1})	98	167	32	85	108	70	55	89
X_m (mg SS L^{-1})	2187	1635	932	1236	1045	930	792	1019
μ (d^{-1})	0.10	0.28	0.44	0.38	0.37	0.42	0.24	0.52
R^2	0.99	0.98	0.98	0.99	0.98	0.98	0.98	0.99

clearly be distinguished; The first group was made up of *Af* with the lowest value obtained 0.1 d^{-1}, The second group of *Sco* and *No* with 0.2 d^{-1} similar to the average specific growth rate obtained by Xin et al. (2010), 0.2 d^{-1} and de Morais and Costa (2007a), 0.26 d^{-1}, for *Sco* cultured in a synthetic modified BG11 growth medium and in Bristol medium, respectively. The third group was *Cv*, Cs and Bb with $\mu \approx 0.4$ d^{-1} values that are analogous to those reported by (Ruiz et al., 2011) for *Cv* cultured in wastewater. And the final group was Ck and the Natural algal bloom with the highest values 0.44 and 0.52 d^{-1} respectively; these results are in line with those reported by Arbib et al. (2013).

Another interesting parameter to consider is the final maximum biomass concentration reached (X_{max}, mg L^{-1}). It can be seen that *Af* and *Sco* reached the highest X_{max} at the end of the tests 2187 and 1635 mg L^{-1} respectively. These results are similar to those obtained by Arbib et al. (2013) ($X_{max} = 1800 \pm 20$ mg L^{-1}) for *Sco* cultivated in urban wastewater but using CO_2 enriched air (5%) ($X_{max} = 1684 \pm 105$ mg L^{-1}). These results indicate that in this work there is no carbon limitation. de Morais and Costa (2007a) also obtained similar X_{max} for *Sco* when cultivated in vertical tubular reactors using CO_2 enriched air in synthetic media ($X_{max} = 1560$ mg L^{-1}). In the case of *Cv,* the X_{max} achieved was lower, 1236 mg L^{-1}. Similar results were obtained by Ruiz et al. (2011) and Arbib et al. (2013) for *Cv* cultivated in urban wastewater, 1249 mg L^{-1} and 1303 mg L^{-1} respectively. On the other hand, the X_{max} for Natural algal bloom, *Cs, Bb*, and *Ck* was around 1000 mgSS L^{-1}. de Morais and Costa (2007a) reported similar results for *Ck* when cultivated in synthetic medium, 980 ± 10 mg L^{-1} with air enriched with CO_2 (6%). Finally, the lowest maximum biomass concentration found was 792 mg L^{-1} by *No*.

It is really important to note that comparison of the specific growth rate (μmax; d^{-1}) and maximum biomass concentration (X_{max}, mg L^{-1}) of *Af* (higher $X_{max} = 2187$ mg SS L^{-1}, and lower $\mu_{max} = 0.10$ d^{-1}) and *Ck* (lower $X_{max} = 932$ mg SS L^{-1}, and higher μmax = 0.44 d^{-1}) will help in understanding the difficulty to compare results between experiments. For this, it is important to use another useful parameter that helps to compare species of microalgae under a unique value that integrates the aforementioned kinetic parameters. This is volumetric productivity.

The volumetric productivity obtained for the seven species and the bloom has been represented in Fig. 3. It can be observed that *bloom*, *Sco* and *Cv* had high productivity rates 118, 108, 99 mg SS L^{-1} d^{-1} respectively, but *No* and *Af* had the lowest productivities at no more than 40 and 44 mg SS L^{-1} d^{-1} respectively. However, there were no significant differences in the rest of the microalgae, which were around 84 ± 7 mg SS L^{-1} d^{-1}. These values were similar to those obtained by other authors for different microalgae species. de Morais and Costa, (2007b) (Sco, 100 mg SS L^{-1} d^{-1}) cultivated in MC medium enriched air CO_2 (6%). Yoo et al. (2010) (*Cv*, 100 mg SS L^{-1} d^{-1}) cultivated in a Watanabe culture medium.

Therefore, we can conclude that in terms of biomass growth and productivity, the *bloom* and *Sco* reported the best results, with the pre-treated wastewater effluents being an appropriate culture medium for this species. The *bloom* is made up of many microalgae species adapted to and proliferate in this medium, as well as being tolerant of indoor conditions. A literature review of wastewater treatment using *Sco* microalgae shows that it has been used in many comparative studies carried out by other authors (Ruiz-Marin et al., 2010; Ruiz et al., 2013a,b; Arbib et al., 2013).

3.2. Nitrogen and phosphorus removal capability

Figs. 4 and 5 show the evolution of total nitrogen (TN) and phosphorus (TP) uptake from culture media by the different microalgae and *bloom* tested.

It can be observed that at the end of the experiments, in all the reactors the removal of TN was higher than 92%, except for *Af, Sco* and *Ck*, in which cases, it was slightly lower (89, 89, and 87% respectively). In the case of *Cs* and *Bb,* dissolved nitrogen in the wastewater was completely removed.

Regarding TP evolution, removal was higher than 90% only for *Cv* and *bloom*, and fluctuated between 79% and 85% for the rest of the species. This data complies with the European Commission Directive 98/15/CE (80% and 70–80% removal for total phosphorus and nitrogen respectively) (the most restrictive percentage in European Union Directive 98/15/CE concerning requirements of N and P in the effluents permitted from urban wastewater treatment).

When applying the PhBT model (Ruiz et al., 2013a,b) (Equation (4)) with the adjustable parameters S_0, Sna, $1/Y_0$ and μN or μP, the coefficient of determination obtained has been higher than 98% in all the experiments, except for Nitrogen for *Sco* and *Ck* (96% and 94% respectively). In the case of phosphorus and the species *Af* and *Sco*, due to the fast initial uptake rate of dissolved phosphorus (Fig. 5), it was not possible to obtain a model.

The removal of nutrients is confirmed by the non-assimilable substrate concentration of nitrogen or phosphorus at the end of the experiments S_{na} (Tables 2 and 3) obtained from the kinetic modelling. This figure is below 10 mgN L^{-1} for all of the microalgae and even negligible amounts (<0.2 ppm) for *Cs* and *Bb* respectively. In the case of phosphorus, S_{na} is between 1 and 2.5 mgP L^{-1} for all microalgae.

As can be seen in Table 2, the highest maximum specific uptake rates for nitrogen (μN; d^{-1}) correspond to *Cs*, *Bb*, and *No*, between 2.11 and 2.38 d^{-1}, followed by *Ck* and *bloom* (1.53 and 1.66 d^{-1} respectively). This is similar to the maximum specific nitrogen uptake rate obtained by Ruiz et al. (2013). On the other hand, *Af*, *Sco* and *Cv* present the lowest values obtained (0.88–1.20 d^{-1}).

Bb and *Ck* presented the highest maximum specific uptake rates for phosphorus μP (2.71 ± 0.04 d^{-1}), and the lowest was *No* (0.46 d^{-1}). The rest of the values fluctuate between 1.81 and 2.31 d^{-1}.

Redfield (1958) reported that uptake rates for phosphorus were always higher than uptake rates for nitrogen, although nitrogen requirements for microalgae are higher. This is consistent with the data reported by Elrifi and Turpin (1985), indicating a luxury consumption for phosphorus four times higher than for nitrogen.

On the other hand, as demonstrated in Ruiz et al. (2013), maximum specific growth rates from biomass (Verhulst model) (μ, Table 1) and from the PhBT model (μN or μP Tables 2 and 3) should be within the same parameters. However, the values obtained for these in the same experiment were different from each other, with

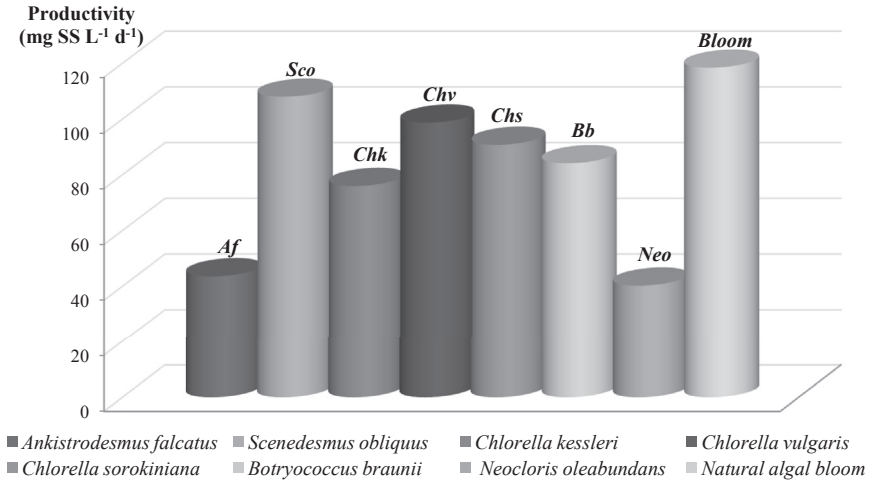

Fig. 3. Biomass batch volumetric productivity (mg SS L^{-1} d^{-1}).

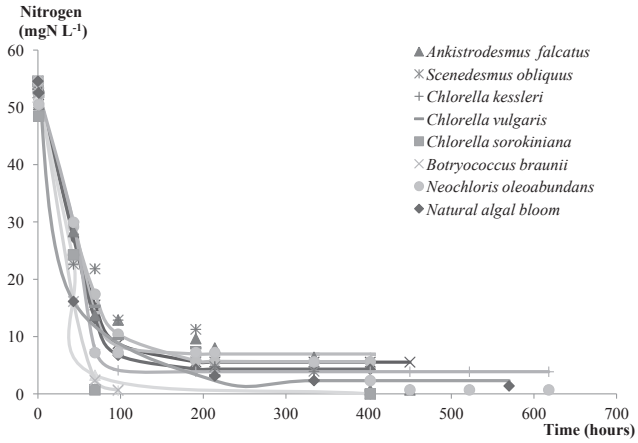

Fig. 4. Modelled and observed dissolved nitrogen concentration evolution. Symbols are experimental data and solid lines represent the predicted concentration of dissolved total nitrogen.

the specific uptake rates for nitrogen and phosphorus (μN or μP) being higher than for biomass growth (μ). The explanation of these higher values for μN or μP is that not all these dissolved nutrients

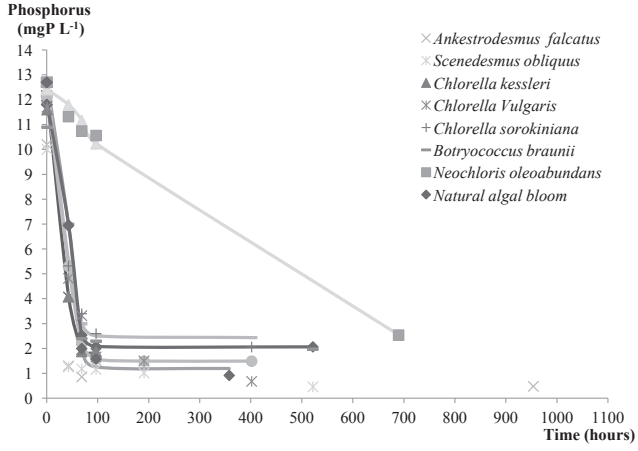

Fig. 5. Modelled and observed dissolved phosphorus concentration evolution. Symbols are experimental data and solid lines represent the predicted concentration of dissolved total phosphorus.

are removed by means of biological processes. An important part could have been removed by means of abiotic processes (stripping of ammonium to ammonia gas or phosphorus precipitation) as both processes occur at pH values of above 9 (Nurdogan and Oswald, 1995; Larsdotter et al., 2007), and the pH during the experiments was higher than 9 in all the tests.

The consumption rate of total nitrogen and phosphorus (dN or dP/dt; mg L^{-1} d^{-1}) (Tables 2 and 3 respectively) will help in the comparison of different microalgae. The dissolved nutrient consumption rate varies throughout the experiment so for comparison purposes, we used the maximum daily consumption rate of nutrients expressed in mg of N or P per reactor volume per day ($\Delta t = 1$ d), using the concentration of nutrients predicted by the corresponding adjusted kinetics model and with Equation (5).

In Table 2, two groups of microalgae can be clearly distinguished. The first group (*bloom*, *No*, *Bb* and *Cs*) characterized by higher maximum daily nitrogen removal rates, between (20–25 mgN L^{-1} d^{-1}), and the rest of the species with lower maximum daily nitrogen removal rates, between (13.49 and 17.14 mgN L^{-1} d^{-1}).

In the case of phosphorus (Table 3), there are fewer differences and the ranking of the species is different from that observed for nitrogen. The lower phosphorus maximum daily uptake rate was found for *Cs* and *Cv* (4.62 and 4.85 mgP L^{-1} d^{-1} respectively) while the higher values were observed in the tests.

3.3. Harvesting

3.3.1. Centrifugation

Fig. 6 shows the efficiency of the centrifugation process at 1000 rpm (G = 96.148) at different times. As can be observed, the efficiency increases with centrifugation time, up to a point where it remains constant, in general at 8–10 min for all the species. But when reducing the time, the efficiency varies significantly and is species-dependent. For example, for 1 min of centrifugation, this fluctuation was between 20% *(Af)* and 79% *(Sco)*. We should note that the initial absorbance of the all of species was the same (O.D. = 1) but the initial concentration was roughly the same for species: *Ck, Cs, No,* and *Af* where it was 0.34 ± 0.02 gSS L^{-1}, and the other group of species had 0.49 ± 0.04 gSS L^{-1} for *Sco, bloom, Cv* and *Bb*. It was assumed that when the biomass concentration was high, the microalgae required more time for harvesting. However, this hypothesis is only true for the same species of microalgae, but when the species changes, the time required for harvesting by

Table 2
Kinetic parameters of total dissolved nitrogen removal obtained from PhBT model (Ruiz et al., 2013a,b).

Parameters	Af	Sco	Ck	Cv	Cs	Bb	No	Bloom
S_0 (mgN L^{-1})	52.54	52.64	52.13	53.18	51.77	53.71	52.60	53.84
Sna (mgN L^{-1})	5.54	5.61	6.97	4.30	0.00	0.38	3.87	2.29
μN (d^{-1})	1.08	0.88	1.53	1.20	2.29	2.11	2.38	1.66
Xo (mgSS L^{-1})	98.48	166.50	32.22	84.66	108.15	70.13	54.62	88.73
R^2	97%	96%	94%	98%	99%	100%	98%	100%
$1/Y_0$ (%N)	10%	10%	10%	10%	1%	4%	1%	10%
$CR_{max}(dN/dt_{max})$ (mgN L^{-1} d^{-1})	15	13.49	17	16.7	27.2	26.7	23.5	21.2

Table 3
Kinetic parameters of total dissolved phosphorus removal obtained from *PhBT* model (Ruiz et al., 2013a,b).

Parameters	Af	Sco	Ck	Cv	Cs	Bb	No	Bloom
S_0 (mgP L^{-1})	–	–	12.17	12.17	12.72	11.81	12.42	12.71
S_{na} (mgP L^{-1})	–	–	1.80	1.49	2.44	2.07	2.55	1.20
μP (d^{-1})	–	–	2.67	1.81	1.82	2.76	0.46	2.31
X_o (mgSS L^{-1})	–	–	32.22	84.66	108.15	70.13	54.62	88.73
R^2	–	–	99%	98%	100%	99%	99%	100%
$1/Y_0$ (%P)	–	–	1.0%	1.0%	1.0%	0.1%	1.0%	0.2%
CR_{max} (dP/dt_{max}) (mgP L^{-1} d^{-1})	11.7*	11.7*	5.9	4.8	4.6	6.7	1.2	6.7

*Data obtained not with modelling but using experiment total dissolved phosphorus concentration.

centrifugation is different and it is dependent on the species. This can be seen in the case of *No*, *Cs* and *bloom* when the initial concentrations were 0.33, 0.35 and 0.52 g L^{-1} respectively and the efficiency after 1 min of centrifugation was 57%, 36% and 36% respectively. It can be observed that for the same concentration (*No* and *Cs*) the efficiency was different and for different concentrations of microalgae (*Cs* and *bloom*) the efficiency was the same. D'Souza et al. (2000) reported that cell viability varied significantly with respect to species, methods of centrifugation and the interaction of the two factors.

Time required for removal of 90% of microalgae is shown as Fig. 7. *Sco* presented the shortest time required to harvest 90% of microalgae (2.9 min), followed by the *bloom* (7.25 min), *Bb* and *Ck (7.7 min)*. *Cs* and *No* obtained this efficiency in 8.7 and 9.3min respectively, while in the case of *Af* and *Cv*, even after the maximum time tested (10 min) 90% of removal was still not reached.

3.3.2. Coagulation–flocculation test

As can be seen in Table 4, different conditions have been used by different authors to test microalgae harvestability by means of coagulation flocculation. Most of them used high volumes of sample (over 150 mL) and different mixing times and intensities. Since the sample volume for jar testing defines the size of the photobioreactors to be used, it is critical in the design of the experiment.

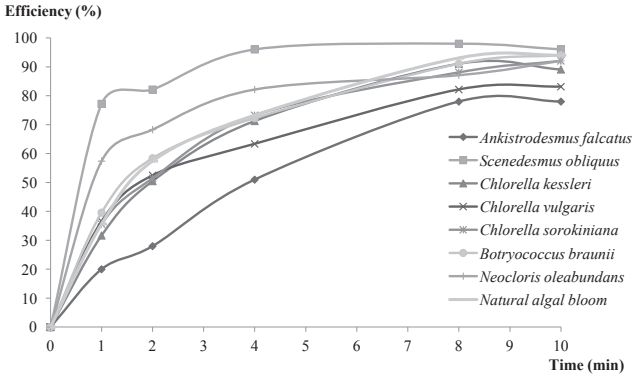

Fig. 6. Harvesting biomass by centrifugation at 1000 rpm.

In order to validate the process of testing microalgae harvesting by means of coagulation–flocculation with a low volume procedure using a tube rotating shaker, both Jar test procedures (micro (10 mL) and standard (1000 mL)) were carried out using *C. vulgaris* cultured in a Standard medium (Combo). As we can see from Fig. 8, both biomass recovery efficiency (BRE) curves showed very similar results. Between 0 and 0.23 mg $FeCl_3$ mg SS^{-1} the curves overlapped one another.

Based on these results, it can be said that this method could be used with great safety for dosages of $FeCl_3$ between 0 and 0.23 mg $FeCl_3$ $mgSS^{-1}$. This method makes it possible to minimize time and reagents as well as sample volume. This last advantage is critical when experiments with low volume photobioreactors are carried out. In these cases the use of a standard Jar test to test harvestability under different conditions implies the use of a large number of reactors while the use of micro jar tests avoids this handicap.

For *Bb*, the BRE obtained (Fig. 9) was higher than the result reported by Kim et al. (2013) (90.6%) where, for the same species cultured in BG11 medium, they found that the optimal conditions inferred from the obtained equation were 0.79 mM (128.14 mg L^{-1}) $FeCl_3$, 0.58% (v/v) bioflocculant obtained from *Paenibacillus polymyxa*, and 180 s of slow mixing for 1.1 g DCW L^{-1} (DCW: Dry Cellular Weight) *B. braunii*. de Godos et al. (2011) reported that the removal of *S. obliquus* exhibited its maximum efficiency at 100 mg $FeCl_3$ L^{-1} (95 ± 3%) but an increase in concentrations of up to 150 and 250 mg $FeCl_3$ L^{-1} severely reduced these removals to 14 ± 9% and 26 ± 2% respectively. In case of *C. sorokiniana*, the maximum BRE was also recorded at 250 mg L^{-1} (66% for $FeCl_3$).

The difference or mismatch in the results can be explained by the differences in the specific nature of aqueous matrices (synthetic media or wastewater) (Jiang et al., 1993), pH, algae concentration, pH (Wyatt et al., 2012) or algae growth phase (Tenney et al., 1969).

On the other hand, it should be pointed out that the addition of ferric chloride decreased the pH from 8.4 to 8.9 in the control tests to 4.4–5.5 at the end of the coagulation–flocculation, except for *No* and *Af*, where this value was 6.80 and 3.58 respectively.

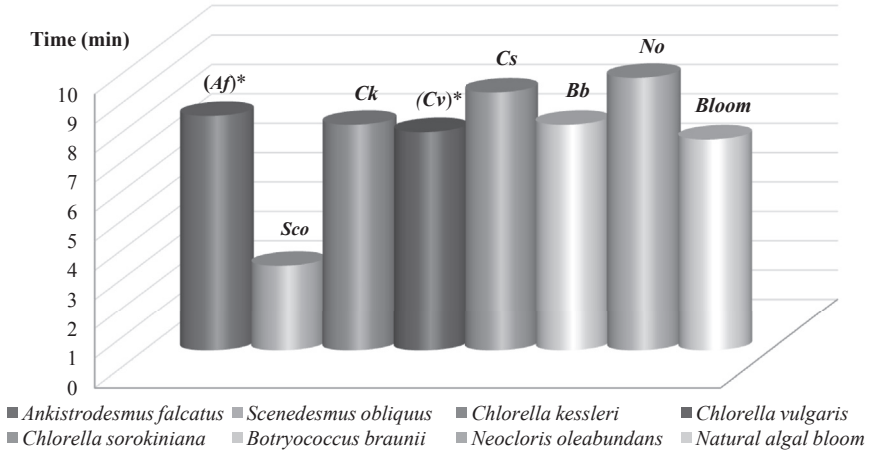

Fig. 7. Time required to remove 90% of microalgae (*78% and 80% for *Af* and *Cv* respectively).

Table 4
Comparison of different coagulation–flocculation test reported in the literature by different authors.

Photobioreactor volume (L)	Glass beakers volume (mL)	Harvest volume (mL)	Coagulation (C)-flocculation (F) settling times (S)	Reference
5 L	1000	700	C: 80 rpm (2 min) F: 20 rpm (30 min) S: 30 min	This study
	12	10	C: 15 s (40 Hz) F: 20 rpm (30 min) S: 30 min	
5 L	100	40	C: 300 rpm (1 min) F: – S: 10 min	de Godos et al., 2011
High rate algal pond (HRAP) 533 L and airlift tubular photobioreactor (TPBR) 380 L	1000	1000	C: 120 rpm (2 min) F: 20 rpm (30 min) S: 30 min	Arbib et al., 2013
1 L (triplicate)	–	500	C: mixed rapidly for 20 s F: slow mixing for 15 min S: 30 min	Xu et al., 2013
5 L (harvesting a 1/3 of the total volume every four days)	500	250	C: 150 rpm (2 min) F: 20 rpm (5 min) S: 15 min	Granados et al., 2012
10 L bubble column photobioreactor	250	150	C: 500 rpm (5 min) F: – S: 2 h	Zheng et al., 2012

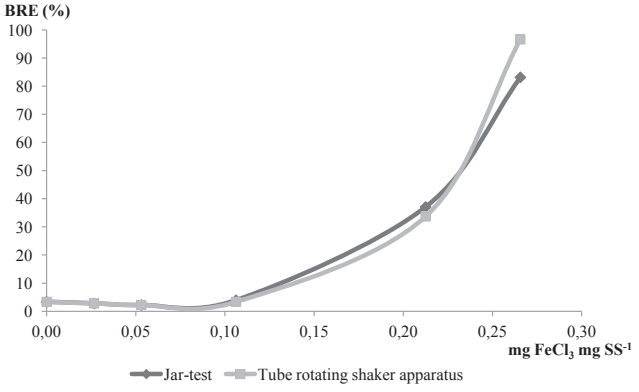

Fig. 8. Effect of $FeCl_3$ dosage on biomass recovery efficiency (BRE) of *Cv* cultivated in a synthetic Combo medium. Comparison between tube rotating shaker procedure (10 mL) and standard jar-test (1000 mL).

3.4. Overall results

As a synthesis of the obtained results, a classification of the species of microalgae has been made according to the result of their biomass productivity, Max daily Nitrogen and Phosphorus removal, harvesting by centrifugation and harvesting by coagulation–-flocculation. This classification was calculated based on the results obtained divided by the highest result obtained for each parameter, except for harvesting by centrifugation, which was divided by the inverse of minimum time value. As can be seen in the normalized data of Table 5, *Sco* presented very good results in terms of biomass productivity, max daily Phosphorus removal, the shortest time required to harvest 90% of microalgae by centrifugation and harvesting by coagulation flocculation but it wasn't the best in terms of max daily Nitrogen removal. *No* was the worst candidate because it presented the lowest results in all of the parameters expect for max daily Nitrogen removal. The results for *natural algal bloom* were also high and it is the best candidate for biomass production. *Bb* was in third place in the classification. The intermediate group was composed of genus of Chlorella (*Cs, Ck* and *Cv*) and *Af* where moderate results were observed.

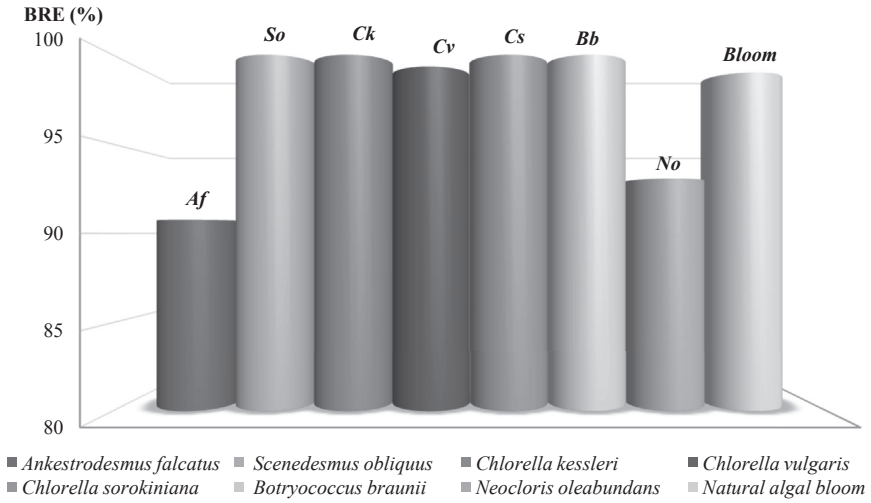

Fig. 9. Effect of 60 mg L^{-1} of $FeCl_3$ dosage on biomass recovery efficiency (BRE) of different species cultivated in wastewater.

Table 5
Microalgae classification.

	Af	Sco	Ck	Cv	Cs	Bb	No	Bloom
Biomass Productivity	0.37	0.91	0.64	0.83	0.77	0.71	0.34	1.00
Max daily Nitrogen removal	0.55	0.49	0.63	0.61	1.00	0.98	0.86	0.78
Max daily Phosphorus removal	1.00	1.00	0.54	0.44	0.42	0.61	0.11	0.61
Harvesting by centrifugation	0.36	1.00	0.38	0.39	0.33	0.38	0.31	0.40
Harvesting by coagulation–flocculation	0.91	1.00	1.00	0.99	1.00	1.00	0.93	0.99
Overall rating	3.19	4.41	3.19	3.27	3.52	3.68	2.55	3.78

4. Conclusions

All the strains tested were able to grow satisfactorily in pre-treated urban wastewater. Significant differences were observed regarding the maximum of the final biomass concentration and specific growth rate between species, with *S. obliquus* and the Bloom reaching the maximum productivity (mg/ld) levels.

Regarding the phytoremediation capability, all species tested have high potential for removing nitrogen and phosphorus from urban wastewater, to levels even lower than the most restrictive laws currently imposed (Directive 98/15/CE).

The proposed low volume harvesting test with a tube rotating shaker showed similar performance to the conventional standard jar test. The proposed test means considerable savings in time and money for both the experiment equipment and the harvestability testing.

Among all the species tested, *S. obliquus* has demonstrated the best performance in all the parameters analysed: biomass production, maximum daily Phosphorus removal, the shortest time required to harvest 90% of microalgae by centrifugation, and the highest efficiency in harvesting by coagulation–flocculation, followed closely by the *Natural algal bloom*.

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